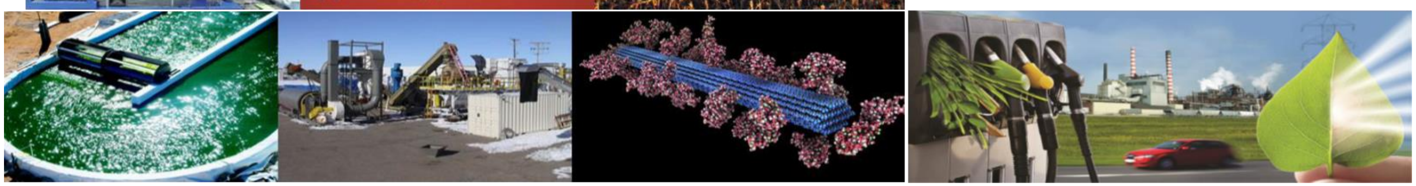
DOE Bioenergy Technologies Office (BETO) 2019 Project Peer Review

Bioconversion of Algal Carbohydrates and Proteins to Fuels



March 5, 2019
Advanced Algae Systems Review

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Goal Statement

- *Goal: Develop cost effective biocatalytic upgrading of the major biochemical fractions of algae biomass to commodity scale chemicals*

- *Expected outcome: Provide capability to produce chemicals from major biochemical components of biomass <u>with market value at parity with costs of algae biomass production and processing</u>*

- *Scale-up of a domestic algae biomass industry requires identification of value propositions for utilization of the bulk of the biomass, not just high value co-products.*

- *If biomass processing options are identified that are synergistic with isolation of high value products and insensitive to compositional detail, risk to investors in expanding algae biomass production are minimized*

Quad Chart Overview

Timeline

- Oct 1, 2018
- Sept 20, 2020
- ~13%

	Total Cost s Pre FY17 **	FY 17 Costs	FY 18 Costs	Total Planned Funding (FY 19-Project End Date)
DOE Funded		$300 k	$250k	$250k
Project Cost Share*				

•Partners: Prof. Varman (ASU), Prof. Quinn (CSU), Prof. (ODU)

Barriers addressed

- AftI: Algal feedstock on-farm processing
- AftJ: Resource recapture and recycle

Objective

Provide capability to produce chemicals from major biochemical components of biomass with market value at parity with costs of algae biomass production and processing

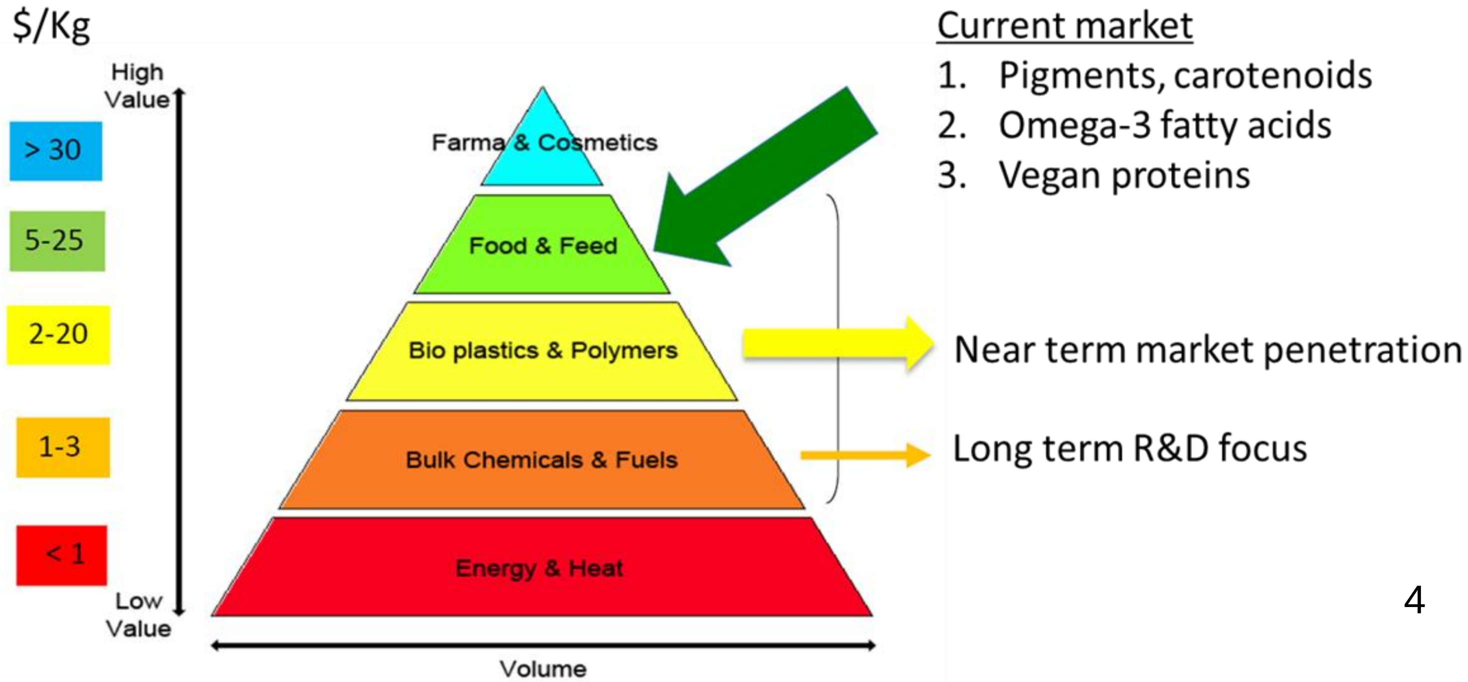
End of Project Goal

Demonstrate bioconversion of low-value biomass to hydroxyalkanoates at 70% mass yield (biomass basis) an titers exceeding 10g/L

Joint TEA for comparison of algal processing targets

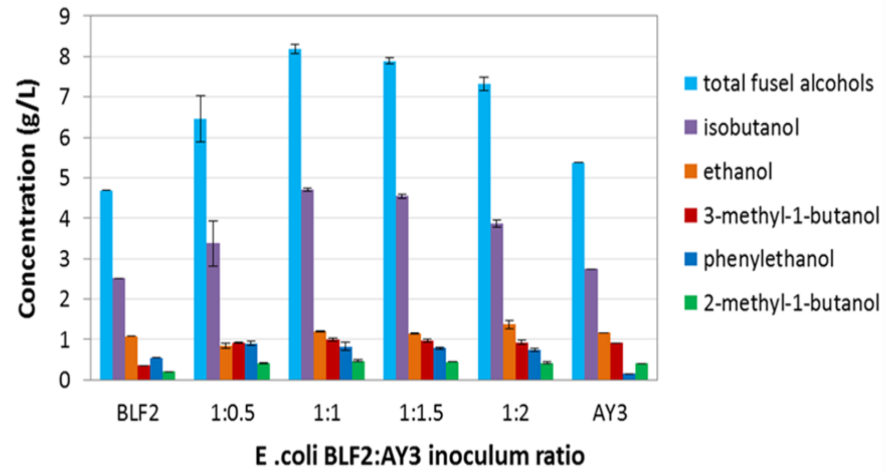
1 - Project Overview

- *Modeled cost of algae production ($500 /ton) + processing ($250 /ton) set minimum value for algae-derived commodity products*
- *Sandia team has identified hydroxyalkanoates as unique opportunity for algae processing based on an expected <u>market value</u> of approx. $800/ton combined with <u>highest potential net yields from both low value amino acids and carbohydrates</u> from bioprocessing (0.93 g/g biomass)*

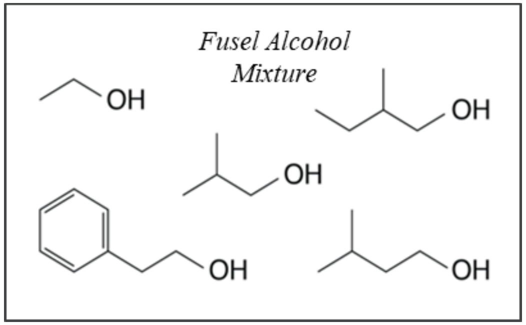


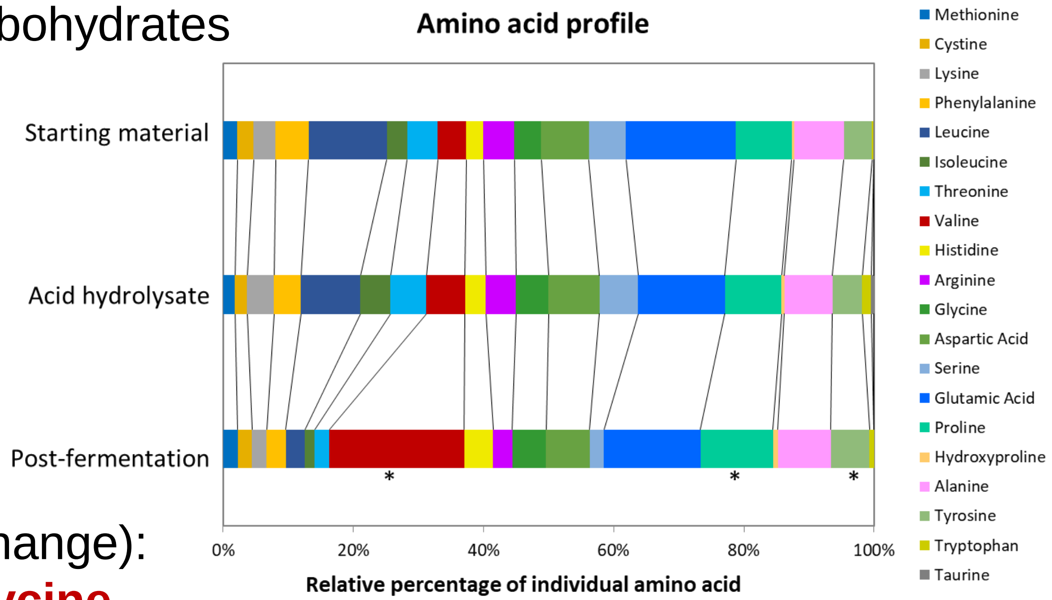
Sandia's initial effort for biochemical algae upgrading

- Based on hypothesis that despite historical focus on lipids, high productivity algae have abundance of protein and carbohydrates



- Utilized biocatalyst consortium for production of <u>fusel alcohols</u> from hydrolyzed proteins and carbohydrates





- Amino acids enriched in the fermentation broth (>15% change): **valine, proline, alanine, glycine, methionine, cystine, histidine, hydroxyproline**

2 – Approach (Management)

- *Provision of biomass in-place through coordination with other BETO-efforts focused on improving yield and decreasing cost of algae production*

- *Sandia staff perform biomass characterization, pretreatment, development of biocatalyst strains, and bench-scale fermentations*

- *Partnering with Prof. Arul Varman (ASU) for metabolic engineering of biocatalyst strains*

- *Partnering with Prof. Sandeep Kumar (ODU) for optimization of solubilization, hydrolysis, and ash-removal from dewatered algae*

- *Partnering with Prof. Jason Quinn (CSU) for independent TEA/LCA of algae bioconversion-derived products, milestone in Q3 2020 for joint TEA/LCA with NREL for assessment of conversion targets*

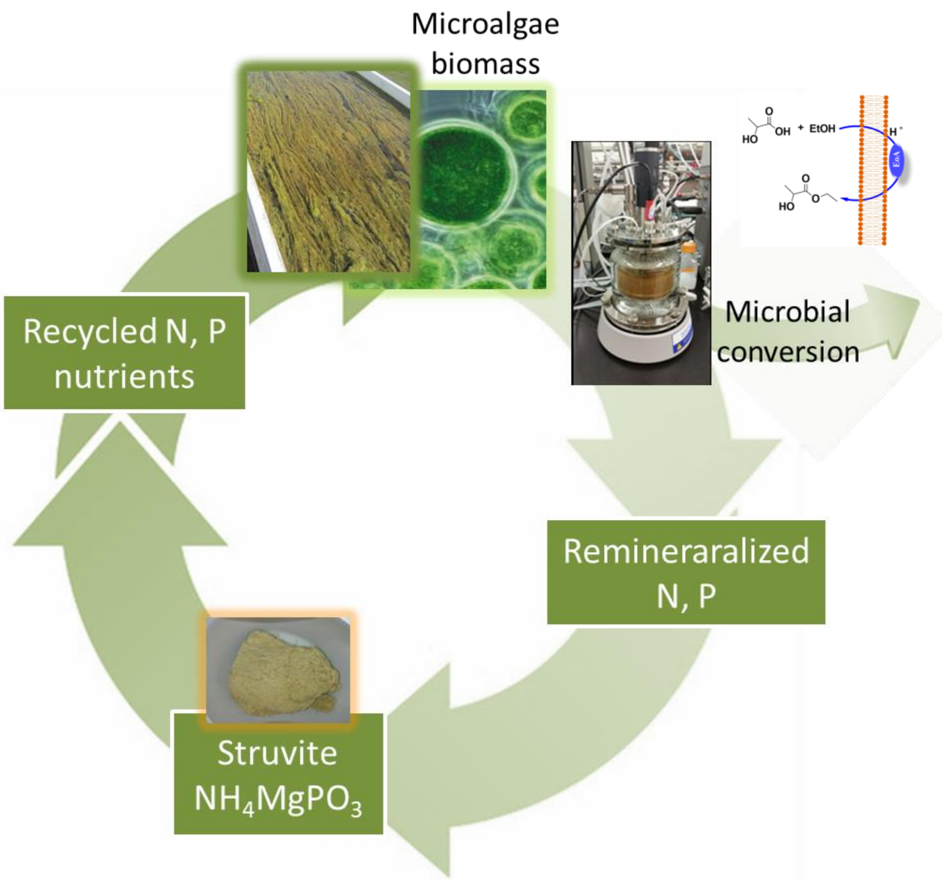
2 – Approach (Technical)

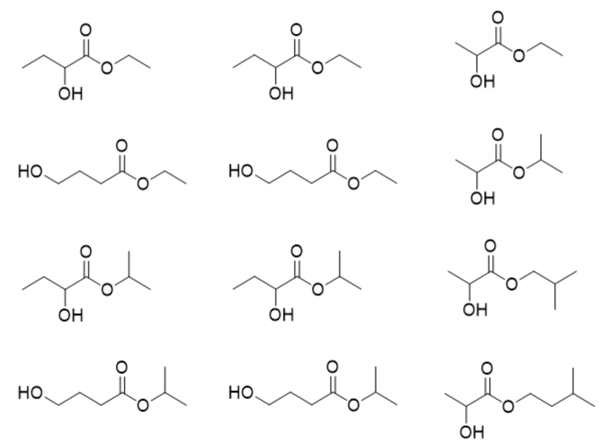
- *Minimize unit operations & intensify process by developing bioconversion consortia for utilization of <u>multi-substrate biomass hydrolysates</u>, i.e. 'single-pot' processing; couple <u>highest net theoretical yield biobased intermediates</u> from amino acid and sugar catabolism to produce chemical commodities*

- Challenges: *Achieve high substrate loadings while minimizing fermentation inhibitors (pretreatment), prevent catabolite repression (metabolism), and utilize moderately specific yet highly active enzymes (biocatalysis)*

- Go/No-Go: *Demonstrate processing yield and titer targets & compare TEA for algae biomass based on projected feedstock costs, processing costs, biofuel intermediate yields, and coproduct contributions*

Underlying biochemistry:
In vivo coupling of protein-derived acids to carbohydrate-derived alcohols to maximize bioprocess yields & minimize unit operations





hydroxyalkanoates

Biodegradable, non-volatile solvent (Kb>1000)
+ predicted to be high octane gasoline blendstock

<u>Yield potential</u>

acid yield from protein/carbohydrates: 0.81 – 0.93 g/g
alcohol yield from carbohydrates: 0.44 g/g
} 0.675 g/g
**alcohol yield from protein: 0.25 g/g

3 – Technical Accomplishments/ Progress/Results

- *Pretreatment: solubilization, hydrolysis, and deashing of wastewater periphytic biomass & raceway cultured microalgae using dilute acid and flash hydrolysis (in collaboration with Prof. Kumar) at near theoretical yield and up to 10% solids*

- *Fermentation: titers of alcohol and acid intermediates >10g/L using biomass hydrolysate; <u>first in vivo production of ethyl lactate </u>using esterase enzyme screening*

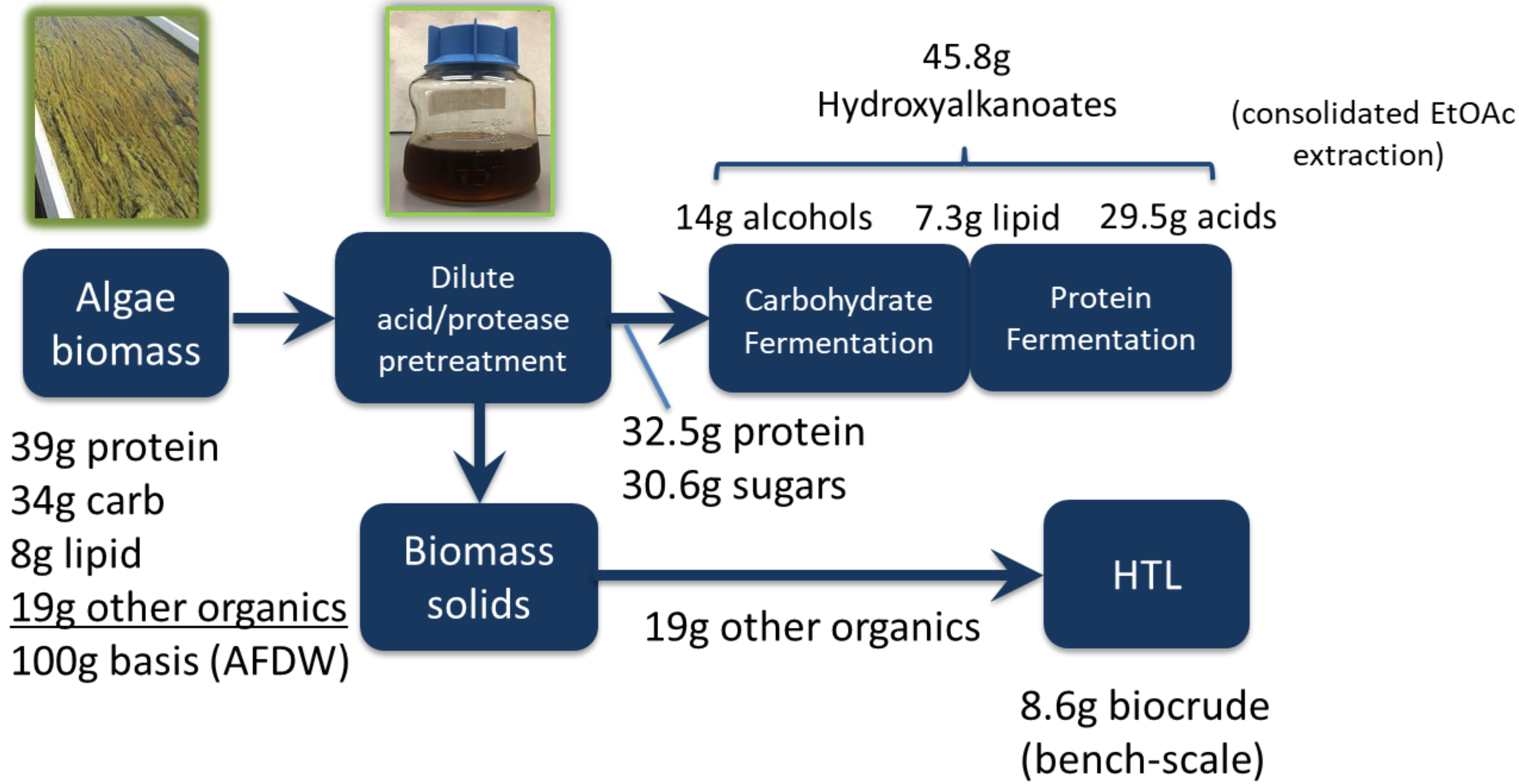
- *In previous effort, we demonstrated C2-C8 fusel alcohol production from algae & other proteinaceous biomass; by targeting hydroxyalkanoates, we can improve yield potential by >2x!*

<u>Yield potential</u>

acid yield from protein/carbohydrates: 0.81 – 0.93 g/g
alcohol yield from carbohydrates: 0.44 g/g
**alcohol yield from protein: 0.25 g/g

} 0.675 g/g

Salton Sea Periphytic Biomass (outdoor ag. runoff polyculture, 15 g m^{-2} day^{-1})



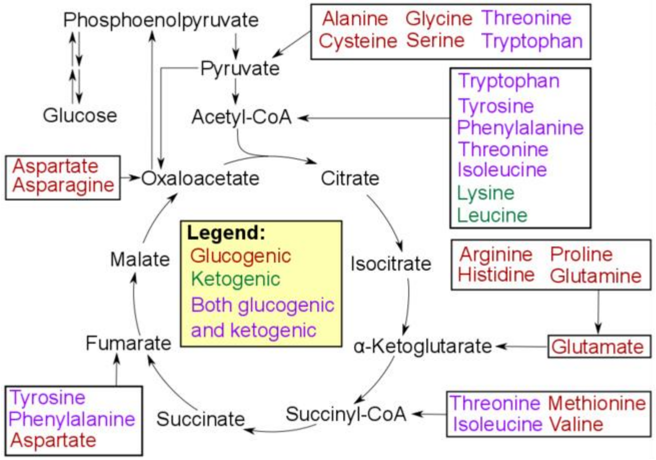
Means to achieve conversion yield that exceed ‘clean’ sugar fermentation to ethanol from crude algae biomass!

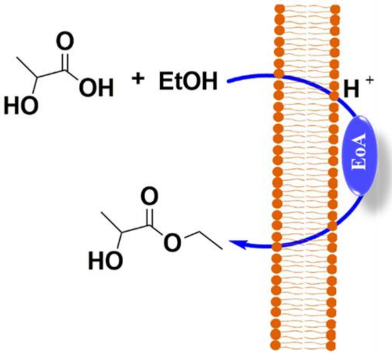
Proof of concept: Ethyl lactate

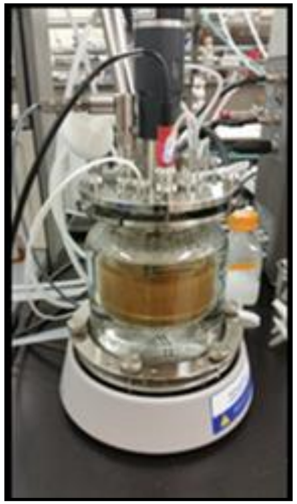
- Utilize *E. coli* bioconversion consortium developed for efficient utilization of proteinaceous biomass

- Esterase enzyme screen for catalyzing condensation of alcohol with lactate

- Identified 4 active enzymes Pid's: EIF47178.1, EIF49674.1, EIF48547.1, EIF45401 which were introduced into ethanologenic *E. coli* and *C. glutamicum;* ethyl lactate product detected by LC-MS *(Dec 2018)*







4 – Relevance

- *Developing means for maximizing economic yield and sustainability of algae biomass production using biochemical upgrading of all of the major conversion substrates to renewable commodity chemicals*

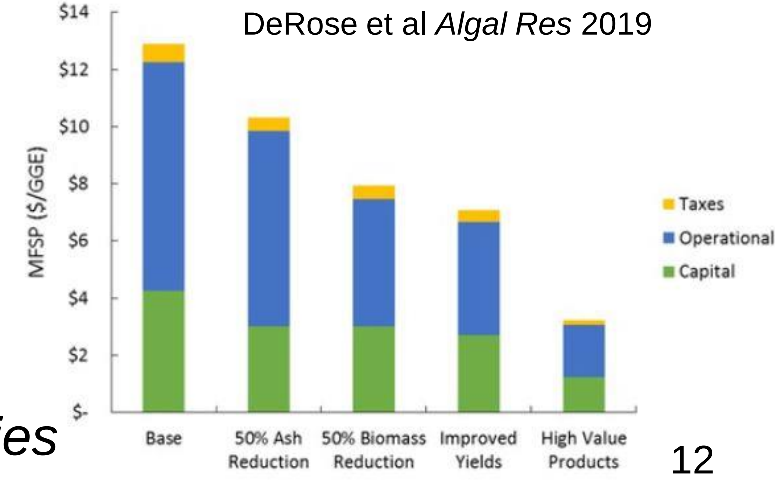
- *Provides value proposition for algae production beyond easily saturated high value markets*

- *On-track to provide capability to produce >3700 gal/acre/year of biofuel intermediate from <u>current algae biomass production yields</u> (20 g/m^2/day biomass productivity) by 2020*

- *Product yield potential is high & is a 'cross-over' chemical, i.e.. can be used as a high performance fuel, if cost can be reduced*

- *IP for proteinaceous biomass being utilized to partner with multiple companies on commercialization opportunities*

DeRose et al *Algal Res* 2019



5 – Future Work

- *Build on ethyl lactate proof-of-concept for in vivo coupling of additional protein derived acids with alcohols*
- *Identify means for product precipitation in anticipation of technoeconomic hurdles associated with separations*
- *Compare modeled MFSP for high protein biomass conversion approaches under development at NREL and SNL based on projected feedstock costs, processing costs, biofuel intermediate yields, and coproduct contributions*
- *Focus on realizing titer, rate, and yield of product suite that was identified in the project development*

Summary

1. Overview: *Provide process to produce chemicals from major biochemical components of biomass with market value at parity with costs of algae biomass production and process compatible with high value co-products*
2. Approach: *Minimize unit operations + maximize mass yield of intermediate value industrial-use oxygenates & 'crossover chemicals' using biocatalyst consortia*
3. Technical Accomplishments: *First demonstration in vivo production of ethyl lactate and achieved >10 g/L titers of intermediates from crude algae hydrolysates*
4. Relevance: *On-track to provide capability to produce >3700 gal/acre/year of biofuel intermediate from current algae biomass production yields (20 g/m^2/day biomass productivity) by 2020*
5. Future work: *achieve necessary TRY (titer, rate, yield); joint TEA/LCA with NREL on near term opportunities for commodity chemicals from algae biomass*

Additional Slides

(Not a template slide – for information purposes only)

- *The following slides are to be included in your submission for Peer Evaluation purposes, but will **not** be part of your oral presentation* –
- *You may refer to them during the Q&A period if they are helpful to you in explaining certain points.*

Responses to Previous Reviewers' Comments

- If your project is an on-going project that was reviewed previously, address 1-3 significant questions/criticisms from the previous reviewers' comments (refer to the 2017 Peer Review Report, see notes section below)
- Also provide highlights from any Go/No-Go Reviews

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Publications, Patents, Presentations, Awards, and Commercialization

- List any publications, patents, awards, and presentations that have resulted from work on this project
- Use at least 12 point font
- Describe the status of any technology transfer or commercialization efforts

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